Award Number: W81XWH-11-1-0365

TITLE: Defining Early Markers of Neurodevelopmental Disorders in Infants With TSC

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REPORT DATE: October 2013

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
October 2013	Annual	15 September 2012 – 14September 2013

4. TITLE AND SUBTITLE

Defining Early Markers of Neurodevelopmental Disorders in Infants With TSC

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-11-1-0365

5c. PROGRAM ELEMENT NUMBER

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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Boston Children's Hospital
Boston, MA 02215

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

In the second year of funding of the "Defining Early Markers of Neurodevelopmental Disorders in Infants with TSC" project, administrative set-up of the collaborative project and IRB approvals were obtained. Post IRB approval (March 28th, 2012) we have successfully recruited 63 families into the project between the two study sites. More specifically, have collected data from 39 participants with TSC as well as 24 age matched-controls between the two performance sites. To date we have completed more than 220 study visits. In keeping with our specific aims, we have begun to collect the data necessary to outline the neurocognitive and behavioral development of children with TSC. With the data that we have collected and evaluated, we have found the tasks that are part of this project appear to be appropriate to describe the pathways to neurodevelopmental disorders in this population.

15. SUBJECT TERMS
None provided.

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
U	U	U	UU	20	19b. TELEPHONE NUMBER *(include area code)*

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INTRODUCTION:

Our goal is to investigate and define clinical, behavioral, and neurophysiological markers of neurodevelopmental disabilities, namely ASD and cognitive impairment, in TSC, prior to clinical diagnosis, which occurs by age 3. Since brain dysfunction is likely to precede abnormal behavior by months or even years, a critical developmental window for early intervention may be missed. Because TSC is often diagnosed prenatally and children are followed clinically throughout their infancy and childhood, a longitudinal study of this kind is not only feasible, but also clinically necessary. We hypothesize that we will be able to detect early neural and clinical markers of cognitive and behavioral deficits in these infants, and that these markers will reflect early aberrant connectivity that occurs prior to the onset of clinical impairment. Furthermore, we hypothesize that it is the dynamic interplay between aberrant functional connectivity and physiological stressors, such as epilepsy, that lead to the heterogeneity of neurodevelopmental outcomes in these children.

BODY:

Specific aims and corresponding tasks taken from the approved scope of work

Specific Aims:

"1. To predict neurocognitive and behavioral outcomes in children with TSC prior to the age of formal clinical diagnosis

> Tasks: Recruit and subsequently evaluate children with TSC and controls to create a behavioral characterization with which to correlate neurocognitive data collected as part of this project. "

In the first funding year we have successfully begun recruiting into the project and, between the two study sites (Boston Children's Hospital, UCLA), have collected data from 39 participants with TSC as well as 24 age matched typically developing controls. We have completed more than 220 study visits. Despite the fact that infants can be challenging to test with EEG and eye tracking, both sites have been very successful in acquiring quality data in these participants. In year one, recruitment was initially delayed due to the time it took to obtain IRB approval from both study sites as well as the funding agency. However based upon our recruitment success within the last year, we anticipate recruitment and retention of the longitudinal sample to proceed as proposed.

"2. To describe pathways to neurodevelopmental disorders in children with TSC, particularly the interaction between clinical factors (such as epilepsy or tuber burden) and cognitive and perceptual domains.

> Tasks: Combine the longitudinal research data collected as part of this grant proposal with available clinical data (such as epilepsy or tuber burden) to evaluate the developmental trajectories of patients with TSC."

All participants who have been consented into this protocol have provided the requested medical record documents and the data have been entered into the study database for use with analyses.

"3. To better define the clinical phenotype of ASD in children with TSC in order to determine if, in fact, the phenotype is distinct from "idiopathic" autism.

> Tasks: Using the clinical assessment of the participants behavior (clinical diagnosis/classification) in conjunction with the neurocognitive assessments completed as part of this proposal (research diagnosis/classification) compare the phenotypic trajectories of those children with TSC who go on to develop autism to those who do not and also to the control groups (neurotypical and infants at-risk). "

Most of our participants have not reached the age of stable behavioral diagnosis, which occurs at 2 or 3 years of age. Currently, only 9 of our TSC subjects have reached their 36 month visit. Based on our current completed 24 and 36 month visits, 15 TSC subjects meet diagnostic criteria on the ADOS for an autism spectrum disorder. Despite the needing to wait for the children to reach this milestone, we have been continually processing data

for analysis, such that when these children reach the age of diagnosis their developmental trajectories can be established. All behavioral testing has been coded and scored, and if there are any red-flags for aberrant development, feedback is provided to the child's primary neurologist.

KEY RESEARCH ACCOMPLISHMENTS:

- Recruitment of patient population
- Recruitment of control population
- Continued data collection
- Data cleaning and entry up to date
- Data analysis and manuscript preparation
- Preliminary graphs of data to evaluate task efficacy provide evidence that tasks are appropriate for the patient population and the specific aims of this project.
- Manuscript published in the Journal for Autism and Developmental Disabilities (JADD)
  Stamoulis, C., Vogel-Farley, V., Degregorio, G., Jeste, S.S., Nelson**,** C.A. (2013, epub). Resting and Task Modulated High-Frequency Brain Measured by Scalp Encephalograms in Infants with Tuberous Sclerosis Complex. Journal of Autism and Developmental Disorders.
- Manuscript published in the Journal of Child Neurology
  Jeste S. S., Hirsch, S., Vogel-Farley, V., Norona, A., Navalta, M. C., Gregas, M. C., & Nelson, C. A. (2012, epub). Atypical face processing in children with Tuberous Sclerosis Complex. Journal of Child Neurology.
- Manuscript submitted to Neurology
  Jeste, S.S., Wu, J.Y., Senturak, D., Varcin, K., Ko, J., McCarthy, B., Shimizu, C., Dies, K., Vogel-Farley, V., Sahin, M., Nelson. C.A. (2013). Cognitive and developmental predictors of ASD in infants with Tuberous Sclerosis Complex.

REPORTABLE OUTCOMES:

- Presentation at the Translational Neuroscience Seminar Series at Boston Children's Hospital, entitled "Cognitive neuroscience and computational radiology approaches to understanding Tuberous Sclerosis Complex", October 2012.
- Manuscript published in the Journal of Autism and Developmental Disabilities entitled "Resting and Task Modulated High-Frequency Brain Measured by Scalp Encephalograms in Infants with Tuberous Sclerosis Complex." This paper analyzed EEG data (baseline and visual task-related) from infants (18-30 months) with TSC and age-matched controls to estimate their respective dominant cortical rhythms encoded in the EEG, and age-related changes in these rhythms. In addition to traditionally estimated rhythms (from delta to gamma), at 18 months a high-frequency (>80 Hz) rhythm was estimated in both cohorts, both at baseline as task-related EEG. This rhythm was no longer present in controls at 24-30 months, but was still present in TSC infants at 24 months, possibly reflection abnormal maturation of cortical circuits in TSC.
    - Specific findings and figures from this paper can be found below.
- Manuscript published in the Journal of Child Neurology entitled "Atypical face processing in children with Tuberous Sclerosis Complex. Journal of Child Neurology." There is a high incidence of autism in tuberous sclerosis complex. Given the evidence of impaired face processing in autism, the authors sought to investigate electrophysiological markers of face processing in children with tuberous sclerosis complex. The authors studied 19 children with tuberous sclerosis complex under age 4, and 20 age-matched controls, using a familiar–unfamiliar faces paradigm. Of the children, 6 with tuberous sclerosis complex (32%) had autism. Children with tuberous sclerosis complex showed a longer N290 latency than controls (276 ms vs 259 ms, P ¼ .05) and also failed to show the expected hemispheric differences in face processing. The longest N290 latency was seen in (1) children with autism and tuberous sclerosis complex and (2) children with temporal lobe tubers. This study is the first to quantify atypical face processing in children with tuberous sclerosis complex. This functional impairment may provide insight into a mechanism underlying a pathway to autism in tuberous sclerosis complex.
- We currently submitted a manuscript to Neurology entitled "Early cognitive and developmental trajectories predict ASD in infants with Tuberous Sclerosis Complex." Drawing on the model of prospective studies of infants at high-risk for ASD, we performed a longitudinal study of infants with TSC, with the overarching goal of defining early clinical, behavioral and biological markers of ASD in this high-risk population. This is the first prospective study of the development of ASD in infants with TSC. Ultimately such investigation should not only shed light on developmental pathways to ASD but also can provide early targets for intervention that could alter the developmental trajectories of these high-risk infants.

- Specific findings and figures from this paper can be found below.

CONCLUSION:

During the second year of our funding, we have continued to successfully collect data for this collaborative project across the two study sites (Boston Children's Hospital & UCLA). A complex administrative structure was established and active recruitment of participants began in March of 2012. The enrollment into the project has been as expected, though the Boston Children's Hospital (n=42) site has had greater numbers in terms of younger infants enrolling into the project. Within the last year, we were able to increase the enrollment of younger infants at the UCLA site (n=21) in order to ensure the age of the participants seen at each site are comparable.

We have evaluated the majority of the data that has been collected in a cross sectional manner to evaluate the task efficacy for this project. We have found that the tasks are appropriate for the age and functional state of the study populations. Within the last year of funding, we have collected enough data to begin analyzing these data longitudinally. Moreover, we have published two manuscripts and have several others in preparation on the data we have collected from this project.

Overall, the specific tasks chosen to address our specific aims are proving to be sensitive to our central research question. Additionally, the multi-site nature of this proposal ensures that we are able to enroll/maintain participants in this longitudinal project.

REFERENCES:

Jeste, S.S., Wu, J.Y., Senturak, D., Varcin, K., Ko, J., McCarthy, B., Shimizu, C., Dies, K., Vogel-Farley, V., Sahin, M., Nelson. C.A. (2013). Cognitive and developmental predictors of ASD in infants with Tuberous Sclerosis Complex (paper has been submitted to Neurology).

Jeste, S.S., Hirsch, S., Vogel-Farley, V., Norona, A., Navalta, M.C., Gregas, M.C. & Nelson, C.A. (2012, epub). Atypical face processing in children with Tuberous Sclerosis Complex. Journal of Child Neurology.

Stamoulis, C., Vogel-Farley, V., Degregorio, G., Jeste, S.S., Nelson**,** C.A. (2013, epub). Resting and Task Modulated High-Frequency Brain Measured by Scalp Encephalograms in Infants with Tuberous Sclerosis Complex. Journal of Autism and Developmental Disorders.

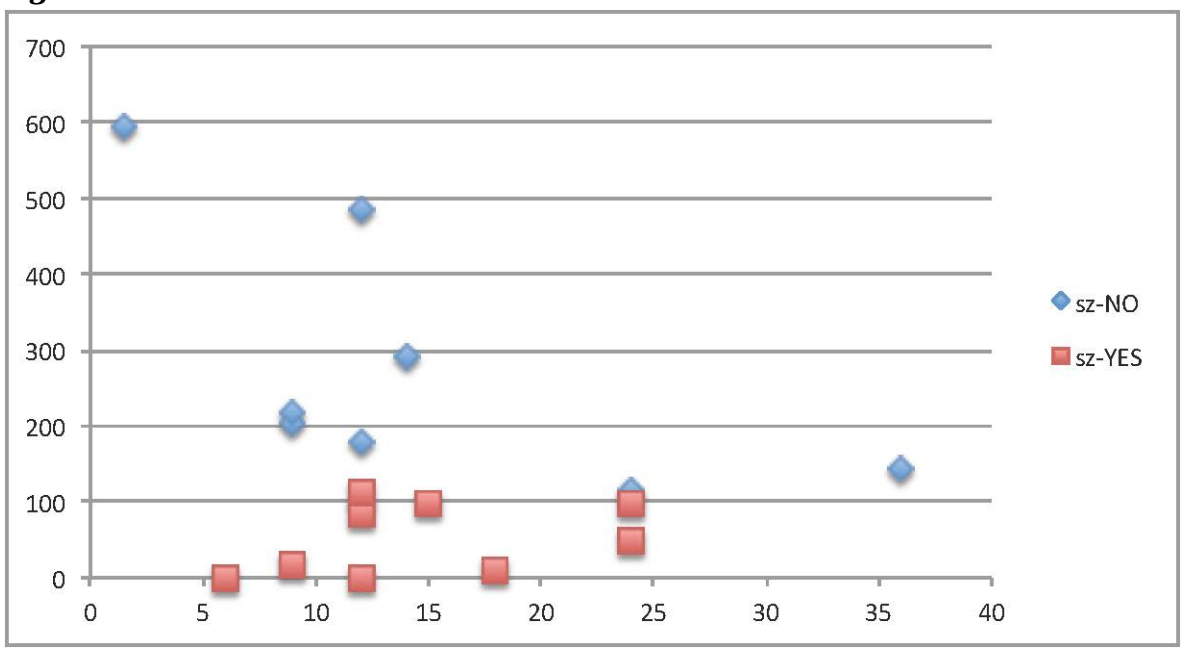
APPENDICES:

None at this time.

SUPPORTING DATA: All figures and/or tables shall include legends and be clearly marked with figure/table numbers.

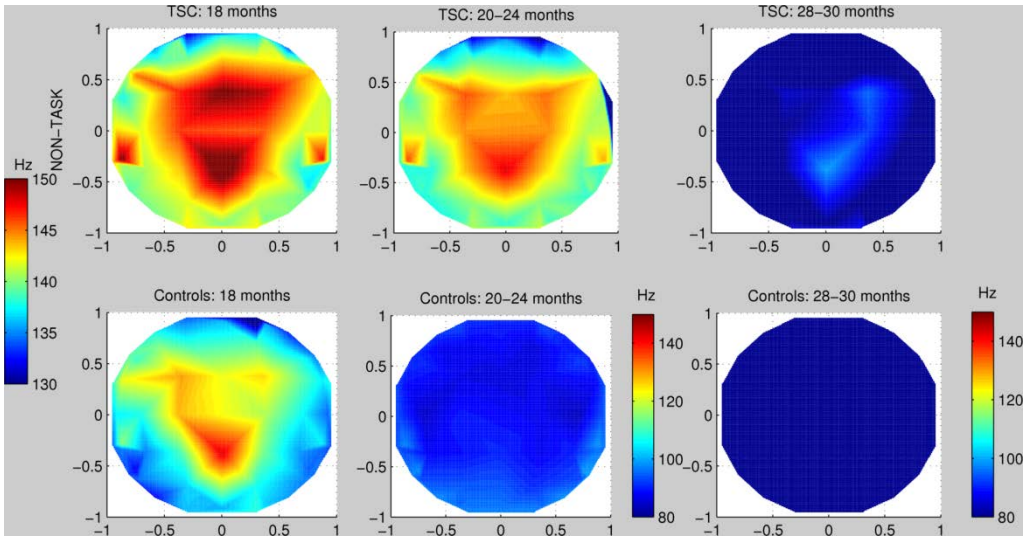
- Preliminary results of EEG analysis indicate the number of scalp-recorded fast ripples correlates with seizure pattern which appear to be higher in the TSC subjects without recent seizures, and lower in the TSC subjects with recent seizures. Furthermore, the delineation between seizure-free and continued seizures appears to be around 100 fast ripples. This finding is illustrated in figure 1 below.

Figure 1



- Y axis is # of high frequency oscillations in the fast ripple bandwidth. X axis is age in months.
- Blue is EEG time points when the subjects have had no seizures since the last visit. Red is EEG time points when the subjects have had seizures since the last visit. A subject could have more than one time point then.

Figure 2:



- Figure 2: Under both baseline and familiar/unfamiliar visual task conditions, there is a high-frequency (>80 Hz) rhythm that is present in both TSC and controls, which disappears by age 20-24 months in controls but is still there in the TSC groups, possibly reflecting aberrant maturation of cortical circuits in TSC.
    - Stamoulis, C., Vogel-Farley, V., Degregorio, G., Jeste, S.S., Nelson, C.A. (2013, epub). Resting and Task Modulated High-Frequency Brain Measured by Scalp Encephalograms in Infants with Tuberous Sclerosis Complex. Journal of Autism and Developmental Disorders.

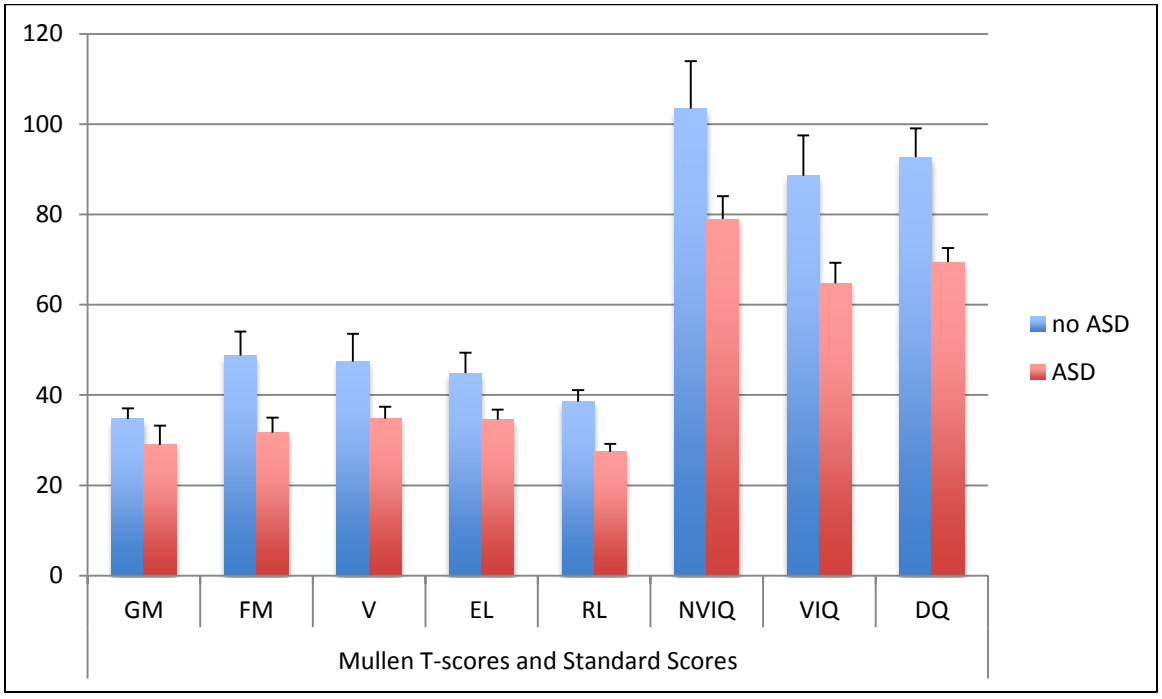
The following figures (Figure 3- 7c) are data from our most recently submitted manuscript to Neurology. Jeste, S.S., Wu, J.Y., Senturak, D., Varcin, K., Ko, J., McCarthy, B., Shimizu, C., Dies, K., Vogel-Farley, V., Sahin, M., Nelson. C.A. (2013). Cognitive and developmental predictors of ASD in infants with Tuberous Sclerosis Complex.

Figure (Table) 3: TSC by ASD grouping – Clinical Variables
Independent samples t-tests comparing markers of seizure severity, including history of spasms, seizures, age of onset, total seizure duration, proportion of life with active seizures and number of anti-epileptics in ASD vs. no ASD.

Table 3. ASD vs. Non-ASD Clinical Variables

	Non-ASD	ASD	p-value
History of Infantile Spasms	66.70%	63.60%	0.51
Seizures	89%	100%	0.11
Seizure Age of Onset (months)	7.46 (5.63)	4.19 (5.41)	0.124
Total Duration of Seizure (months)	10.30 (9.58)	16.29 (9.26)	0.08
Percent of Life with Seizure	38.46 (37.33)	57.16 (32.04)	0.13
AED number	1.14 (.86)	1.79 (.92)	0.05

Figure 4: TSC by ASD grouping – 12 month MSEL
Independent samples t-tests comparing MSEL T-scores and Standard Scores by ASD grouping. Means and standard error bars are provided, with asterix signifying significance of $p<0.05$.



Figures 5 (a-c): TSC/ASD vs. TSC/no-ASD developmental trajectories of Nonverbal IQ (NVIQ) from MSEL

Figure 5a: Individual DQ plots
Individual plots of DQ in all children followed longitudinally from 12-36 months

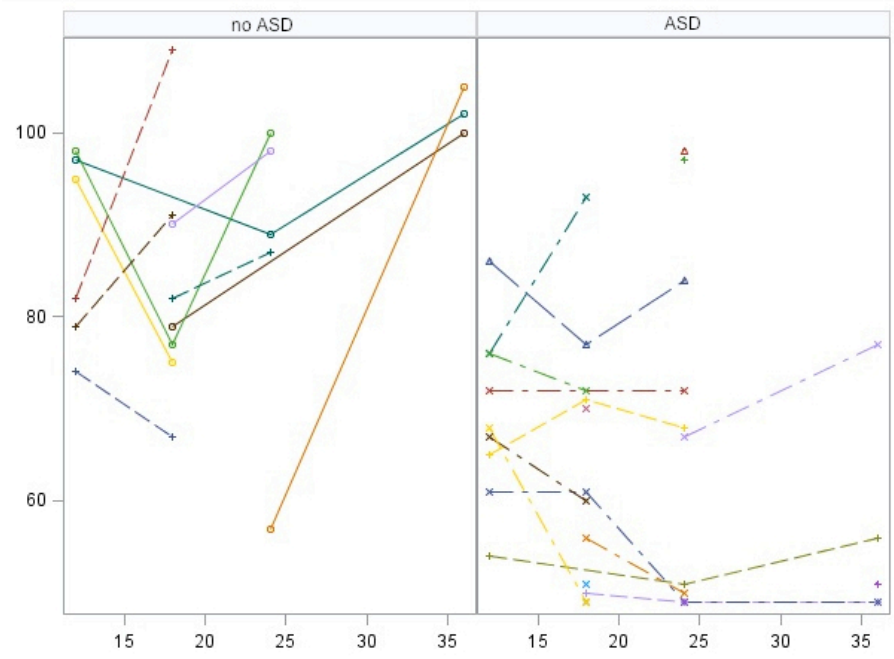


Figure 5b: Mean population DQ trajectory (raw data)
Group plots (ASD vs. no-ASD) of DQ from 12-36 months

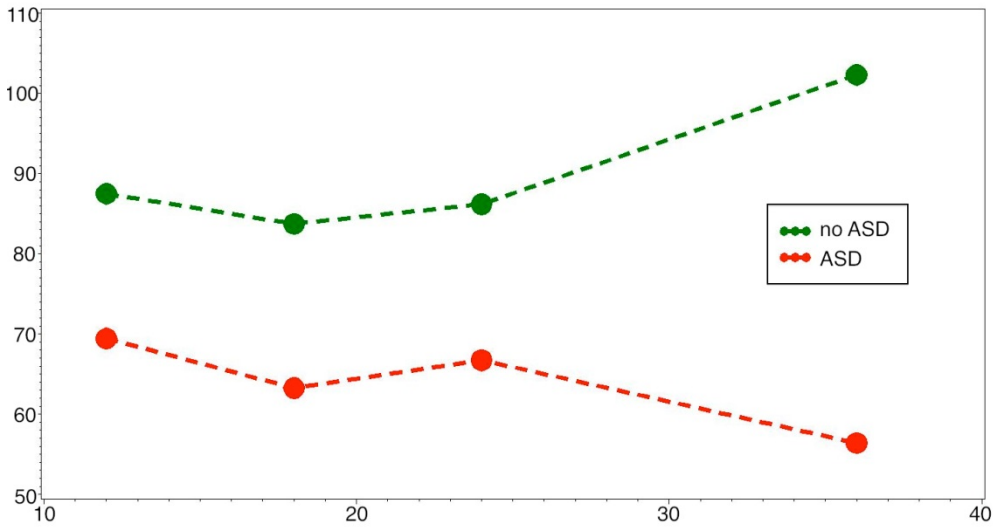


Figure 5c: Regression statistics
Statistics from linear mixed effects models using group, time, group by time interaction, seizure duration, and group by seizure duration interaction as predictors

IQ trajectory controlling for seizure duration (proportion of life with sz)

Effect	Estimate	Standard Error	DF	P value
Intercept	81.88	9.81	21	<0.001
Time	-0.22	0.33	25	0.51
ASD = 0	-14.87	14.94	21	0.33
Time*ASD	1.25	0.52	25	**0.02**
Percent sz	-0.19	0.11	21	0.10
Percent sz*ASD	0.17	0.16	21	0.29

Figures 6 (a-c): TSC/ASD vs. TSC/no-ASD developmental trajectories of Nonverbal IQ (NVIQ) from MSEL

Figure 6a: Individual NVIQ plots
Individual plots of NVIQ in all children followed longitudinally from 12-36 months

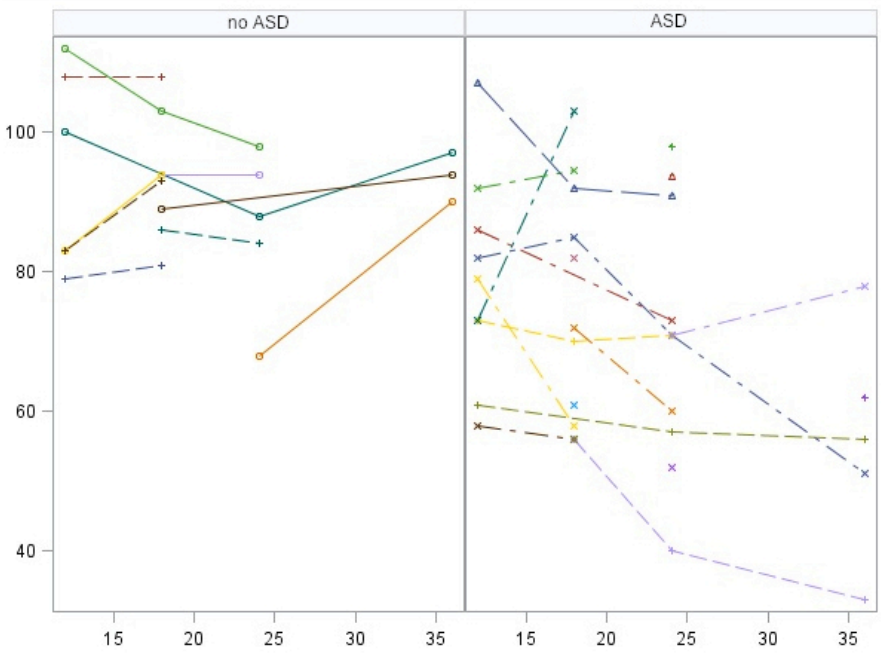


Figure 6b: Mean population NVIQ trajectory (raw data)
Group plots (ASD vs. no-ASD) of NVIQ from 12-36 months

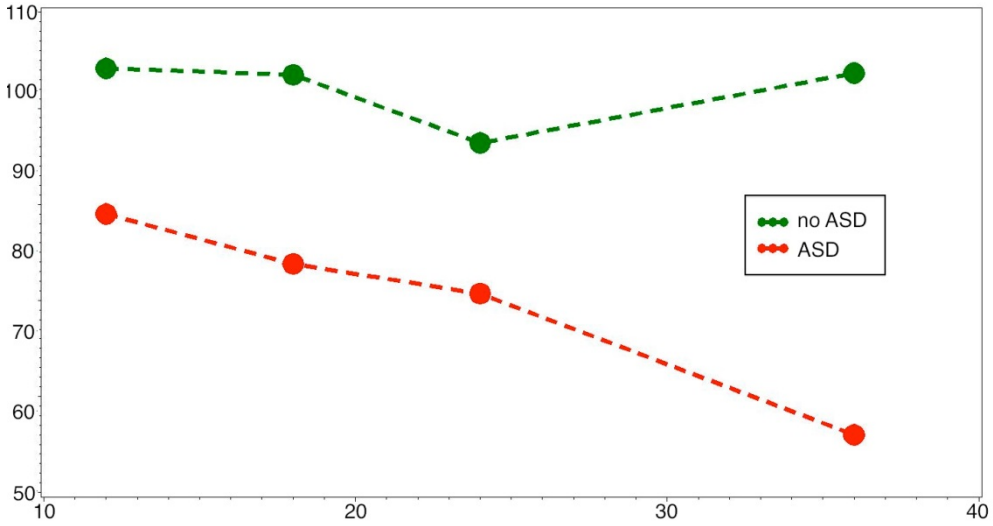


Figure 6c: Regression statistics
Statistics from linear mixed effects models using group, time, group by time interaction, seizure duration, and group by seizure duration interaction as predictors

NVIQ trajectory controlling for seizure duration (proportion of life with sz)

Effect	Estimate	Standard Error	DF	P value
Intercept	103.64	9.37	21	<0.001
Time	-0.80	0.29	25	**0.01**
ASD = 0	-23.03	14.02	21	0.12
Time*ASD	1.22	0.46	25	**0.01**
Percent sz	-0.25	0.11	21	**0.03**
Percent sz*ASD	0.27	0.16	21	0.10

Figures 7 (a-c): TSC/ASD vs. TSC/no-ASD developmental trajectories of Verbal IQ (VIQ) from MSEL

Figure 7a: Individual VIQ plots
Individual plots of VIQ in all children followed longitudinally from 12-36 months

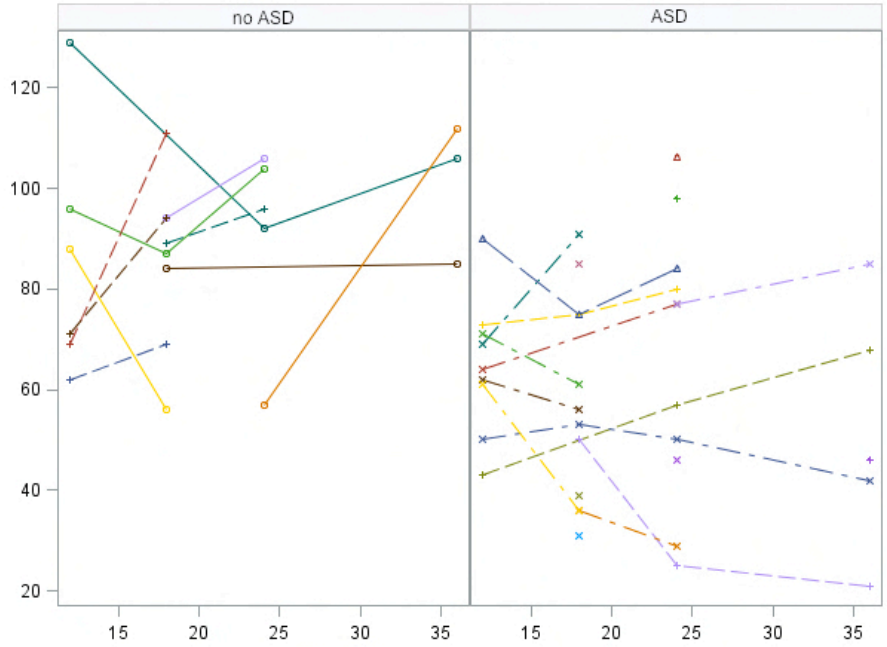


Figure 7b: Mean population VIQ trajectory (raw data)
Group plots (ASD vs. no-ASD) of VIQ from 12-36 months

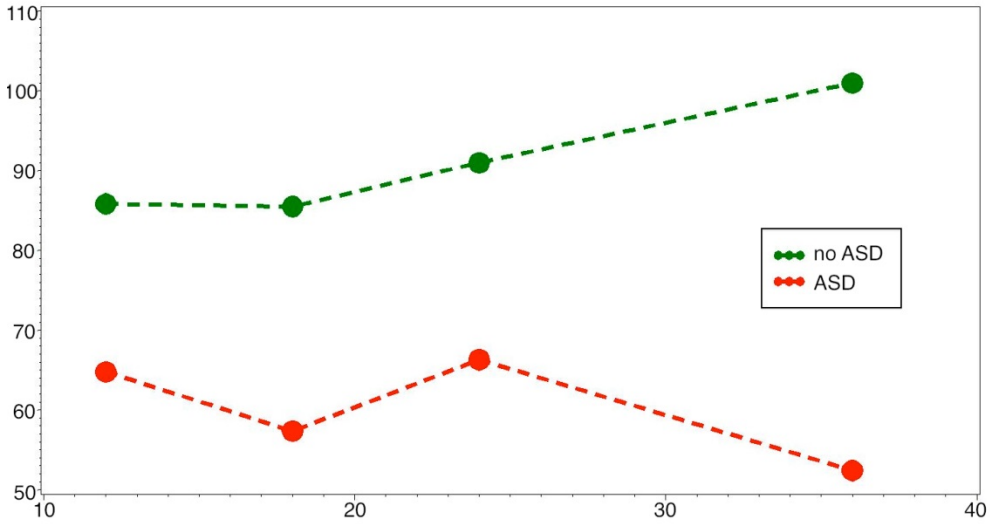


Figure 7c: Regression statistics
Statistics from linear mixed effects models using group, time, group by time interaction, seizure duration, and group by seizure duration interaction as predictors

VIQ trajectory controlling for seizure duration (proportion of life with sz)

Effect	Estimate	Standard Error	DF	P value
Intercept	81.33	14.44	21	<0.001
Time	-0.22	0.50	25	0.66
ASD = 0	-3.11	22.12	21	0.89
Time*ASD	1.04	0.78	25	0.19
Percent sz	-0.25	0.16	21	0.13
Percent sz*ASD	0.08	0.23	21	0.73